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BRAIN'S STRESS AXIS: A POSSIBLE PREDICTOR FOR THE QUALITY OF MENTAL PERFORMANCE DURING CHRONIC STRESS

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13. ABSTRACT (Maximum 200 words)
There has been no major change in teh research objectives proposed earlier. Briefly, through the research outlined in this proposal we had hoped to achieve following specific goal. To develop a biochemical measure that will predict the quality of mental performance under conditions of acute and chronic stress. To identify such a predictor, we will examine the relationship between the secretion of adrenal as well as 'adrenal-modulated' hormones (dehydroepiandrosterone, corticosterone, progesterone, pregnenolone and allopregnenolone) and a variety of behavioral tests for emotionality and anxiety, and cognition under normal and stress conditions. During the course of this study, however, we observed that under stress-free conditions, rats exhibit a wide animal-to-animal variation in two of the behavioral measures, the Porsolt's test for despair and the Elevated-plus maze test for anxiety, that we had proposed to use. Therefore, we have decided to characterize the nature of this variation first and then look for biochemical predictors in different subsets of animals. Of all the adrenal hormones (dehydroepiandrosterone, corticosterone, progesterone, pregnenolone and allopregnenolone) considered, DHE appeared to be very promising. Therefore, the rest of the studies were largely focussed on DHEA and its role in stress anxiety.

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FINAL TECHNICAL/INVENTION REPORT

1. COVER SHEET

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2. OBJECTIVES:

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During the course of this study, however, we observed that under stress-free conditions, rats exhibit a wide animal-to-animal variation in two of the behavioral measures, the Porsolt's test for despair and the Elevated-plus maze test for anxiety, that we had proposed to use. Therefore, we have decided to characterize the nature of this variation first and then look for biochemical predictors in different subsets of animals. Of all the adrenal hormones (dehydroepiandrosterone, corticosterone, progesterone, pregnenolone and allopregnenolone)) considered, DHEA appeared to be very promising. Therefore, the rest of the studies were largely focussed on DHEA and its role in stress anxiety.

3. STATUS OF EFFORT:

(1) MODULATION OF GABA-GATED CHLORIDE ION INFLUX IN THE BRAIN BY DEHYDROEPIANDROSTERONE AND ITS METABOLITES.

Both DHEA and $GABA_A$ receptor agonists are known to reduce anxiety. Since $GABA_A$ receptor agonists are generally thought to elicit their anxiolytic effects by facilitating neuronal uptake of chloride ion, we set out to evaluate whether DHEA elicits its anxiolytic effects by a similar mechanism. The results of the studies show an uneven distribution of basal and GABA-stimulated chloride uptake in different regions (cerebellum, pons-medulla, striatum, hippocampus, mid-brain, hypothalamus and cortex) of rat brain. Contrary to our expectations, however, both DHEA and DHEAS inhibited GABA-mediated chloride uptake with DHEAS being more potent than DHEA. On the other hand, Δ^4-androstenedione, another DHEA metabolite, did not have any effect on chloride uptake in any region of the brain. In conclusion, the data presented here, therefore, suggest that DHEA and DHEAS may elicit anxiolysis through mechanisms independent of $GABA_A$ receptor-mediated facilitation of neuronal chloride uptake.

(2) INCREASED GABA-GATED CHLORIDE ION INFLUX IN THE HYPOTHALAMUS OF LOW-ANXIETY RATS.

While it is generally accepted that $GABA_A$ receptor agonists decrease anxiety by facilitating the neuronal influx of chloride, the site of action within the brain is not clearly delineated. To gain further insight into the locus of anxiolytic action of

GABA in the brain, we measured the distribution of GABA-stimulated chloride influx in seven regions of the brain from high- and low-anxiety rats. Our results show a significant increase in GABA-gated chloride influx in the hypothalamus of rats exhibiting low anxiety. The role of the hypothalamus in the regulation of anxiety is briefly discussed.

(3) Heterogeneity In The Response Of Outbred Sprague-Dawley Rats To Anxiolytic Effects Of Diazepam.

Outbred S-D rats exhibit considerable heterogeneity within a population when evaluated for expression of anxiety response (AR) in the elevated-plus maze test. For example, the naive rats show a wide variation in AR, and on successive testings, the AR increases for some and decreases or remains unchanged for others. These results open the possibility of developing an animal model for better screening of anxiolytic and anxiogenic agents. Forty naive rats underwent 3 successive testings in the elevated-plus maze. The results showed that 45% (18/40), 40% (16/40), and 15% (6/40) fell into high-to-high (H-H), low-to-high (L-H), and low-to-low (L-L) categories, respectively (H= high and L= low anxiety). The first letter in the H-H, L-H and L-L designations signifies the result of the first test; the second letter indicates the result of the repeat test. Three weeks later, animals in the above three groups were tested once in the maze after vehicle (corn oil) and the next day after diazepam (D), an anxiolytic drug. There was significant (p=0.019) reduction in the AR after D (5 mg/Kg, IP) when the AR of all animals was evaluated together. However, when the response of the three subgroups (H-H, L-H, and L-L) was analyzed separately, a different pattern emerged. D caused a significant reduction in the L-H (p=0.002) but not the H-H group (p=0.089). In contrast, D led to a significant increase in AR (p=0.031) in the L-L. In conclusion, variations in the AR in outbred rats could be used to develop better screening for AR-modulating agents. *This research was supported by the AFOSR (DEPSCoR-94-NL-102).*

(4) DEHYDROEPIANDROSTERONE DECREASES BEHAVIORAL DESPAIR IN HIGH- BUT NOT LOW-ANXIETY RATS.

Outbred S-D rats exhibit considerable heterogeneity within a population when evaluated for a variety of biologic functions such as dietary fat intake, alcohol preference, and expression of anxiety. To understand the neuroendocrine basis for depression and anxiety, we routinely assess outbred rats for behavioral despair (Porsolt test), anxiety (elevated-plus maze), and urinary excretion of a variety of hormones. In one such study, we observed a significant correlation (r^2=0.337, n=30, p<0.01) between the level of anxiety and the degree of behavioral despair. Within this population two distinct subgroups emerged: one (12/30) with high anxiety and high despair (HA/HD), the other (10/30) with low anxiety and high despair (LA/HD). We next evaluated the effect of DHEA, an anxiolytic neurosteroid, on the

despair response in the two groups of rats. Twelve HA/HD and 10 LA/HD rats were divided equally into 2 groups (control or C and DHEA or D-treated). On day 1, all rats underwent Porsolt test 30 min after vehicle or V administration (1 ml/Kg, ip). On day 2, C group received V and D group DHEA (1 mg/Kg, IP), and both were again subjected to Porsolt test. D treatment of HA/HD (day 1 vs day 2: V/V, p=0.942, V/D, p=0.026) , but not LA/HD (day 1 vs day 2: V/V, p=0.758, V/D, p=0.750) rats significantly diminished behavioral despair. The preliminary results presented here show D to be effective as an anti-despair agent in rats exhibiting both a high degree of anxiety and high degree of despair.

4. ACCOMPLISHMENTS/NEW FINDINGS:

The data summarized above show the following major accomplishments or new findings. These include,
(i) Outbred Sprague-Dawley rats vary widely in their endogenous levels of anxiety. About 45%, 40%, and 15% rats fall into high-to-high (H-H), low-to-high (L-H), and low-to-low (L-L) categories, respectively (H= high and L= low anxiety).
(ii) The above three groups of rats respond differently to diazepam, an anxiolytic agent. This information will greatly facilitate our ability to understand the nature of biochemical predictors for stress.
(iii) DHEA, an adrenal androgen (also known as one of the many neurosteroids) may serve as a marker for stress. DHEA and its sulfated metabolite, $DHEA.SO_4$, decrease GABA-mediated chloride ion influx into neurosynaptosomes. These data suggest that it is unlikely that anxiolytic actions of DHEA is mediated through GABA receptor.
(iv) It was interesting to report that GABA-mediated chloride influx was much higher in the hypothalamus of low-anxiety rats compared to those expressing elevated anxiety. Therefore, one of the brain sites where GABA acts to regulate anxiety response may be hypothalamus.

5. PERSONNEL SUPPORTED:

Makoto Imamura, MD

6. PUBLICATIONS:

Prasad, Amit and C. Prasad (1996). Short-term consumption of a diet rich in fat decreases anxiety in rats. Physiol. & Behav. 60:1039-1042.

Prasad, Amit, M. Henry, and C. Prasad (1996). Heterogeneity in the Performance of Outbred Sprague-Dawley Rats in an Elevated-Plus Maze Test: A Possible Animal Model for Anxiety Disorder. Life Sci 59: 1499-1506.

Prasad C., Amit Prasad, and Makoto Imamura (1997). Dehydroepiandrosterone decreases behavioral despair in high- but not low-anxiety rats. Physiol & Behav 62:1053-1057.

Imamura, M and C, Prasad (1998) Modulation of Gaba-Gated chloride ion influx in the brain by dehydroepiandrosterone and its metabolites. Biochem. Biophys. R e s . Commun (In Press).

Imamura, M and C, Prasad (1998) Increased GABA-gated chloride ion influx in the hypothalamus of low-anxiety rats. Physiol & Behavior (Under review).

7. INTERACTIONS/TRANSITIONS:

Abstracts presented at scientific meetings:
(1)   Prasad, Amit, Prasad, C., and Henry, M. (1995). Heterogeneity in the Performance of Outbred Sprague-Dawley Rats in an Elevated-Plus Maze Test: A Possible Animal Model for Anxiety Disorder. Abstract Clinical Research.
(2)   Amit Prasad, Makoto Imamura, Anoop Prasad, and C Prasad, Dehydroepiandrosterone (DHEA) decreases behavioral despair in rats selected for mixed despair and anxiety, but not despair alone. Abstract Clinical Research.
(3)   Imamura, M. and C. Prasad (1997) Differential modulation of GABA-gated chloride ion influx in the rat brain by DHEA and its metabolites. Soc. Neurosc. Abstract # 382.11, p. 961.

8. NEW DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES:

NONE

9. HONORS/AWARDS:

Dr. Prasad was appointed to serve as Editor-in-Chief of Nutritional Neuroscience - In International Journal on Diet, Nutrition and the Nervous System.